ANTI-PCSK9 MONOCLONAL ANTIBODY

TECHNICAL FIELD

[0001] The disclosure relates to the technical field of antibody engineering, and in particular to a fully human anti-Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) monoclonal antibody, obtaining method and application thereof.

BACKGROUND

[0002] Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) belongs to a proteinase K subfamily of proprotein convertase. The human PCSK9 gene is located at chromosome 1p32.3, has a length about 22 kb, has 12 exons and is capable of encoding a protein having 692 amino acid residues. The PCSK9 protein is composed of a signal peptide, a front structural domain, a catalytic domain and a carboxyl terminal structural domain (V structural domain), is synthesized as a soluble precursor of 74 kDa, and is capable of generating propeptide of 14 kDa and mature protease of 60 kDa by means of catalytic cracking of self in an endoplasmic reticulum. The PCSK9 is mainly expressed in livers, intestinal tracts and kidneys, and is also slightly expressed in skin and nerve systems, but only the PCSK9 in the livers can be secreted into blood circulation systems.

[0003] Research shows that the PCSK9 is capable of mediating degradation of a Low Density Lipoprotein. Receptor (LDLR) to regulate the level of Low-Density Lipoprotein-Cholesterol (LDL-C) in plasma, and the LDL endocytosis process mediated with LDLR in liver is a main way for eliminating LDL from circulatory system. The LDLR is a protein having multiple structural domains, and its extracellular domain is tightly connected with epidermal growth factor precursor homologous structural domains EGF-A, EGF-B and EGF-C. When degradation of the LDLR is mediated with the PCSK9, the PCSK9 firstly needs to be bound with the LDLR, the LDLR mainly has a binding site which is mainly EGF-A, and a composition of the PCSK9 and the EGF-A is formed. Research shows that the PCSK9 is also capable of regulating cholesterol metabolism by means of a very low density lipoprotein receptor, an apolipoprotein B receptor and an apolipoprotein E receptor, but molecular mechanisms therein are not clear.

[0004] Basic study and clinical test show that inhibiting activity of the PCSK9 by means of ectogenic interference measures, elimination of Low Density Lipoprotein (LDL) in the plasma can be accelerated, and thus a blood fat reduction function can be achieved. At present, PCSK9 inhibitors mainly include monoclonal antibodies, antisense nucleotides, small interfering Ribonucleic Acid (RNA), mimic peptides, small-molecule inhibitors, and the like.

[0005] The monoclonal antibody medicine is a research and development hotspot of a biomedicine field in the year, which has characteristics of being good in targeting property, high in specificity, low in toxic or side effect, and the like, This represents a latest development direction of a medicine treatment field. A monoclonal antibody having the PCSK9 as a target can be specifically combined with the PCSK9 and is capable of interdicting interactions of the PCSK9 and the LDLR and retarding a degradation process of the LDLR so as to take an effect of reducing the level of LDL-C. The clinical experimental data showed the safety, effectiveness and unique clinical disclosure values of anti-PCSK9 monoclonal antibody medicine.

[0006] A fully human antibody is a main direction of the development of therapeutic antibodies. Due to an antibody library technique, a good technical platform is provided for preparation and screening of human antibodies. Due to the antibody library technique, an essential hybridoma process in a conventional monoclonal antibody research process is avoided, and even various antibody genes and antibody molecular fragments can be obtained without an immunologic process. The phage antibody library is the earliest and most widely used antibody library at present. According to the source of antibody genes, the phage antibody library is divided into an immune library and a nonimmune library, and the nonimmune library also includes a natural library, a semisynthesis library and a complete synthesis library. An antibody affinity maturation process is simulated in screening of the phage antibody library, generally an antigen is coated by a solid phase medium, a phage antibody library to be screened is added, and multiple rounds of processes "adsorption, washing, elution and amplification" (that is, elutriation) are carried out till an antibody having high affinity specificity is screened.

[0007] At present, multiple pharmaceutical companies are actively developing monoclonal antibody medicine targeting at PCSK9. Repatha (evolocumab) of Amgen and Praluent (alirocumab) of Sanofi/Regeneron are both fully human antibodies, are approved to sell in 2015 successively and are applied to treat on primary hypercholesterolemia and familial hypercholesterolemia (heterozygote and homozygote). On the basis of statin, the LDL-C of a patient suffering from primary hypercholesterolemia can be reduced by 77% together with Evolocumab, the LDL-C of a patient suffering from heterozygote familial hypercholesterolemia can be reduced by 68%, and the LDL-C of a patient suffering from homozygote familial hypercholesterolemia can be reduced by 31%. The evolocumab has good tolerance and has no conspicuous security problem at present. A human monoclonal antibody bococizumab of Pfizer is at phase-III clinical test, and a human monoclonal antibody lodelcizumab of Novartis is at phase-II clinical test. Roche and Merck are also having clinical test.

[0008] At present, China is still in lack of self-developed anti-PCSK9 fully human antibodies having high affinity in the field.

SUMMARY

[0009] The disclosure provides an anti-Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) monoclonal antibody. Anti-PCSK9 monoclonal antibodies are screened from a complete synthesis antibody library; a small-capacity synthetic phage antibody light chain library is established by means of computer aided design and analysis; a library of mutations of light chain Complementarity-Determining Regions CDR1, 2, 3 of the anti-PCSK9 monoclonal antibodies is obtained by means of screening; after screening, monoclonal antibodies having high affinity are selected; a library is established to screen mutations at heavy chain regions CDR1, 2, 3 of the monoclonal antibodies; and finally an anti-PCSK9 monoclonal antibody having high affinity is obtained by means of screening. The anti-PCSK9 monoclonal antibody has completely new sequences, has good functions in vitro, particularly at a cellular level, and has very good medicinal disclosure prospects.